Automating Rapid High-throughput mAb Attribute Screening of Microbioreactor Cell Culture Media Samples

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INTRODUCTION

- Rapid and high-throughput screening of monoclonal antibodies (mAb) can potentially streamline their development
- Implementation of laboratory automation for routine analysis of biotherapeutics can improve data reliability and laboratory efficiency
- A high-throughput automated analytical platform combining a compact pipetting robot and benchtop TOF LC-MS for routine analysis of antibody from cell culture media is presented
- Small sample volumes (20-100 µL) of the mAb media samples were evaluated for this study either directly (unpurified media) or as Protein A purified samples for subunit, and possibly peptide or glycan level analyses

EXPERIMENTAL METHODS

Chemicals	Materials	Volume
Cell Culture Media	Filtered CHO Cells Culture Spent Media	100 µL
Magnetic Beads	Promega Magne™ Protein A Magnetic Affinity Beads	50 µL
Equilibration Buffer	1X Phosphate Buffer Saline (PBS), pH 7.4	3 × 150 µL
Wash Buffers	1X PBS pH 7.4 and Water	3 × 150 µL
Elution Buffer	Glycine-HCl, 200 mM, pH 2.5	2 × 50 µL
Neutralization Buffer	MES 100 mM and Tris-HCl 900 mM pH 7.5	60 µL
Enzyme	Ides 2 unites/µL in water	15 µL
Reducing agent	DTT in Guanidine-HCl 6 M and Tris-HCl 200 mM	35 µL

LC System:	ACQUITY UPLC I-Class Plus
Detection:	TUV detector (280 nm)
Column:	BioResolve™ RP mAb Polyphenyl Column 450 Å, 2.7 µm, 2.1 mm x 100 mm P/N 176004157
Column Temp.:	80 °C
Sample Temp.:	10 °C
Injection Volume:	3 µL
Flow Rate:	0.4 mL/min
Run Time:	4.5 minutes
Mobile Phase A:	0.1% formic acid in water
Mobile Phase B:	0.1% formic acid in acetonitrile

LC Gradient

Time (min)	Flow Rate (ml/min)	A (%)	B (%)
0	0.4	80	20
2.00	0.4	59	41
2.20	0.4	15	85
2.30	0.4	15	85
2.50	0.4	80	20
3.50	0.4	59	41
3.60	0.4	15	85
3.65	0.4	15	85
3.80	0.4	80	20
4.50	0.4	80	20

MS System	ACQUITY RDa™
Ionization Mode	ESI+
Acquisition Range	50-2000 m/z
Capillary Voltage	1.50 kV
Scan Rate	2 Hz
Cone Voltage	30 V
Lock-Mass	waters_connect™ Lockmass solution

Data acquisition and processing software	waters-connect with INTACT Mass ™

RESULTS AND DISCUSSION

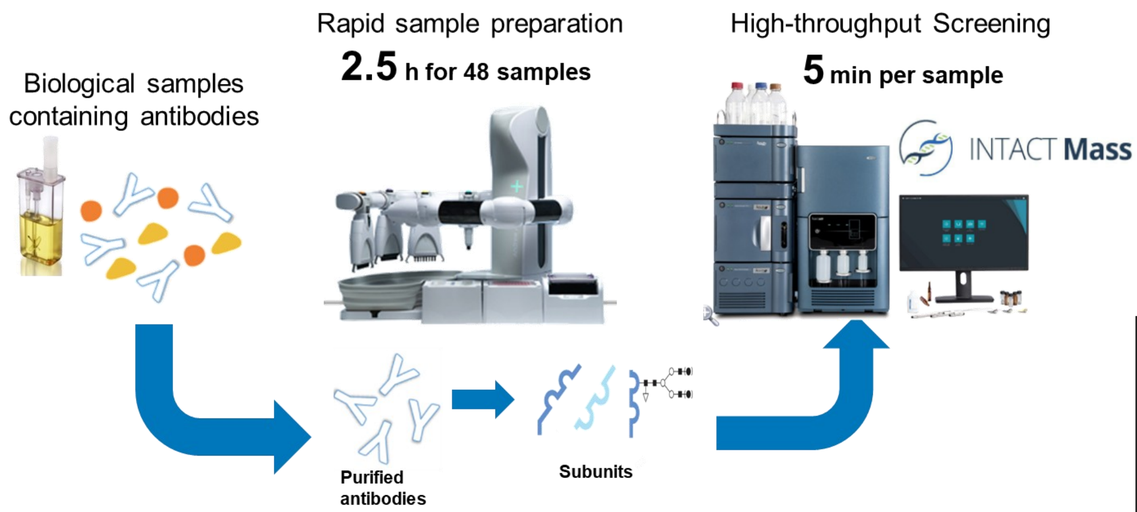


Figure 1. Rapid high-throughput mAb subunit screening workflow

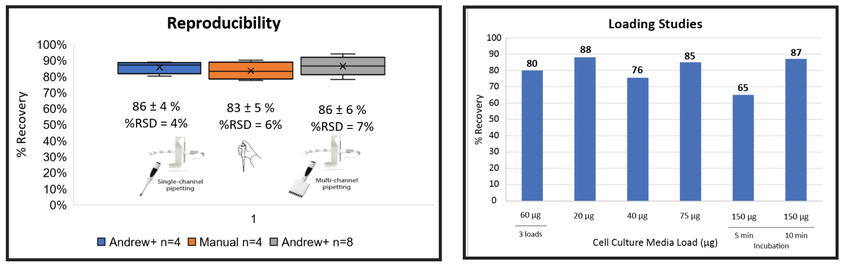


Figure 2. Reproducibility of mAb recovery for automated purification protocol

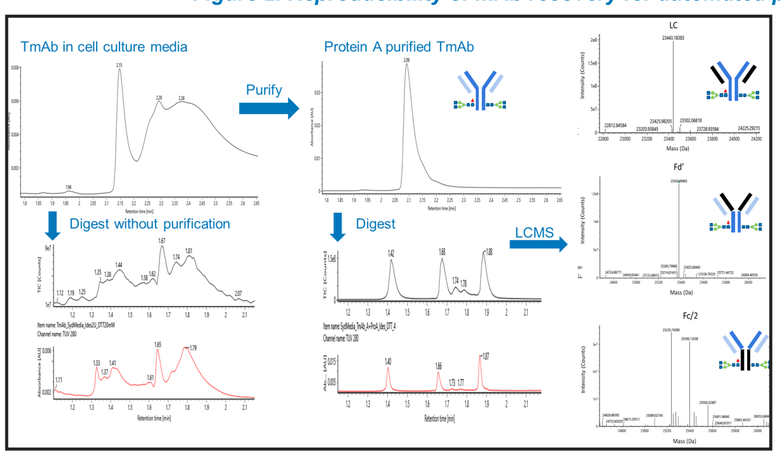


Figure 3. LC-MS analysis of protein A purified digested mAb

Subunits (n = 8)	Control Average Subunits Mass (Da)	Manual Average Subunits Mass (Da)	Andrew+ Average Subunits Mass (Da)
LC	23443.26 ± 0.03	23443.18 ± 0.05	23443.19 ± 0.05
Fd'	25383.39 ± 0.07	25383.13 ± 0.09	25383.59 ± 0.07
Fc/2			
• G0	25089.67 ± 0.08	25089.30 ± 0.16	25089.04 ± 0.13
• G0F	25236.05 ± 0.05	25236.07 ± 0.11	25236.02 ± 0.09
• G1F	25398.37 ± 0.06	25398.30 ± 0.12	25398.46 ± 0.08
• G2F	25560.82 ± 0.12	25560.64 ± 0.26	25560.14 ± 0.26

Figure 4. Reproducibility of selected mAb subunit mass

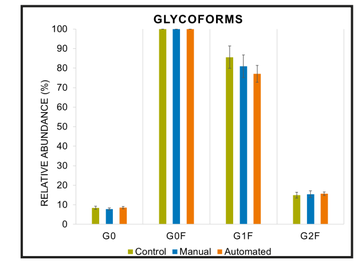
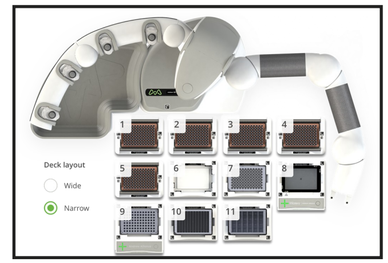


Figure 5. Reproducibility of relative glycan abundance determinations



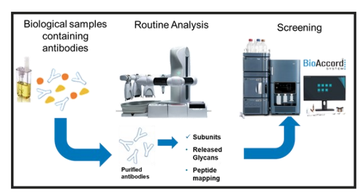
Position	Dominos and connected devices
1, 2, 3, 4, 5	Tip Insertion System Domino
6, 7	Microplate Domino
8	Plate Heater-Shaker+
9	96-PCR Plate Magnet+
10, 11	Deepwell Microplate

Figure 6. Andrew+ domino configuration for automated subunit protocol. 2.5 h for 48 samples

CONCLUSION

- Rapid turnaround time for sample preparation and analysis
  - 2.5 h for 48 samples on Andrew+ Pipetting Robot™
  - 5 min per LC-MS analysis on BioAccord™
- Easily modifiable OneLab™ automated protocol works with a broad range of sample titers during the cell cloning process (0.5 mg/ml or higher)
- Low sample volume (100 µL) requirement for automated protocol is compatible with microbioreactors
- Automated data acquisition and processing in Unifi™ for rapid mass confirmation of subunits on BioAccord

FUTURE DIRECTIONS



References

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2. *Htet, Y.; Koza, M. S.; Chen, W. Chen. 720007343, Waters Corporation August 2021.*
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